Amendments to the Specification:

Please replace paragraph **[03]** beginning at page 1, line 16, with the following:

--**[03]** Surface expression of proteins via covalent linkage with peptidoglycans in Gram-positive bacteria involves unique sorting signals and Sortase-dependent machinery (Mazmanian *et al.*, Science 285:760-763 (1999)). One of the best-studied systems is the *emm6* gene of *Streptococcus pyogenes* that encodes the M6 structural protein (Fischetti *et al.*, 1990. *Mol. Microbiol.* 4:1603-1605 (1990)). The M6 proteins have a signature cell wall sorting signal, the Leu-Pro-X-Thr-Gly (LPXTG; SEQ ID NO:9) motif, followed by a stretch of hydrophobic amino acids and finally a sequence containing charged residues (KRKEEN; SEQ ID NO:10), which serves as a cell surface retention signal. These cell wall sorting motifs have been identified in other Gram-positive bacteria including *Staphlyococcus*, *Enterococcus*, and *Listeria*, and *Lactobacillus* (Navarre and Schneewind, *Microbio. Mol. Biol. Rev.* 63:174-229 (1999)), but not in *Lactobacillus* species that colonize the human vagina.--

Please replace paragraph **[11]** beginning at page 3, line 6, with the following:

--**[11]** In some embodiments, the cell wall targeting region comprises the amino acid sequence LPQSG (SEQ ID NO:11). In some embodiments, the cell wall targeting region comprises the amino acid sequence LPQAG (SEQ ID NO:12). In some embodiments, the cell wall targeting region comprises the amino acid sequence LPQTG (SEQ ID NO:13). In some embodiments, the cell wall targeting region comprises the amino acid sequence LPQTA (SEQ ID NO:14). In some embodiments, the cell wall targeting region comprises SEQ ID NO:7. In some embodiments, the cell wall targeting region comprises SEQ ID NO:8.--

Please replace paragraph **[19]** beginning at page 4, line 14, with the following:

--**[19]** In some embodiments, the cell wall targeting region comprises the amino acid sequence LPQSG (SEQ ID NO:11). In some embodiments, the cell wall targeting region comprises the amino acid sequence LPQAG (SEQ ID NO:12). In some embodiments, the cell wall targeting region comprises the amino acid sequence LPQTG (SEQ ID NO:13). In some embodiments, the cell wall targeting region comprises the amino acid sequence LPQTA (SEQ ID NO:14). In some embodiments, the cell wall targeting region comprises SEQ ID NO:7. In some embodiments, the cell wall targeting region comprises SEQ ID NO:8.--

Please replace paragraph **[31]** beginning at page 6, line 3, with the following:

--**[31]** In some embodiments, the cell wall targeting region comprises the amino acid sequence LPQSG (SEQ ID NO:11). In some embodiments, the cell wall targeting region comprises the amino acid sequence LPQAG (SEQ ID NO:12). In some embodiments, the cell wall targeting region comprises the amino acid sequence LPQTG (SEQ ID NO:13). In some embodiments, the cell wall targeting region comprises the amino acid sequence LPQTA (SEQ ID NO:14). In some embodiments, the cell wall targeting region comprises SEQ ID NO:7. In some embodiments, the cell wall targeting region comprises SEQ ID NO:8. In some embodiments, the biologically-active polypeptide is between 10 and 600 amino acids.--

Please replace paragraph **[53]** beginning at page 12, line 16, with the following:

--**[53]** Figure 1 illustrates the structures of three cell wall anchored proteins identified after genomic sequencing of *L. jensenii* 1153. All of the three proteins have LPQTG (SEQ ID NO:13) sorting signal preceding a hydrophobic region and a charged C-terminal tail and possess unique long repetitive sequences. CWA represents putative cell wall associated regions upstream of the LPQTG (SEQ ID NO:13) motif.--

Please replace paragraph **[54]** beginning at page 12, line 21, with the following:

--**[54]** Figure 2A-C illustrates cell wall anchor sequences (C14 (SEQ ID NO:1), C191 (SEQ ID NO:2), and C370 (SEQ ID NO:3)) resulting from genomic sequencing of *L. jensenii* 1153. The CWA200 region along with anchor motif is underlined. CWA200 represents putative cell wall associated or spanning regions of about 200 amino acids upstream of the LPQTG (SEQ ID NO:13) motif.--

Please replace paragraph **[59]** beginning at page 13, line 20, with the following:

--**[59]** Figure 7 illustrates the surface expression of 2D CD4 in *L. jensenii* 1153 as affected by different number of the repetitive cell wall spanning sequence upstream of the LPQTG (SEQ ID NO:13) sorting signal in C370 sequence. Surface exposed 2D CD4 molecules that adopt a correctly folded conformation were probed with mAb Sim.4 for flow cytometric analysis in the bacterial cells harboring the following plasmid: 175, a negative control; 249, two and a half repeats; 262, no repeat; 268, one repeat; 278, two repeats; 280, four repeats; 281, seven repeats; 276, eight repeats.--

Please replace paragraph **[60]** beginning at page 13, line 27, with the following:

--**[60]** Figure 8 illustrates the surface display of c-Myc tagged proteins in a variety of lactobacillus species of human origin. (A). Schematic of pOSEL241 designed for expression of c-Myc tagged CWA200 of C370 sequence under control of P23 promoter and CbsA signal sequence (CbsAss). cNyc epitope EQKLISEEDL = SEQ ID NO:15. (B). Western analysis of cell wall enriched fractions following mutanolysin digestion of transformed *L. jensenii*, *L. gasseri*, and *L. casei*. After separation in reducing SDS-PAGE, the proteins were electroblotted to PVDF membrane for probing with mAb against c-Myc. (C). Flow cytometric analysis of human vaginal lactobacillus isolates harboring pOSEL241. The bacterial cells were

probed with mAb against c-Myc, and then phycoerythrin (PE)-conjugated anti-mouse antibodies. Controls consisted of unstained cells or cells probed with PE-conjugated secondary antibodies.--

Please replace paragraph **[61]** beginning at page 14, line 3, with the following:

--**[61]** Figure 9 illustrates the effect of point mutations in the LPQTG (SEQ ID NO:13) motif of C14 and C370 sequences on the surface display of 2D-CD4-CWA200 in *L. jensenii* 1153. Bacterial cells were surface-stained by using pre-titered mAb Sim.4 (A) or pAb T4-4 (B), followed by probing with PE-conjugated anti-mouse or FITC conjugated anti-rabbit antibodies. The flow cytometric analysis was performed in a FACScalibur system. The difference between the protein displayed on the cell surface of pOSEL237, pOSEL249, and those in bacterial cells harboring mutagenic constructs was expressed in mean fluorescence intensity. The surface display of 2D CD4 in the bacterial cells harboring pOSEL237 or pOSEL249 was arbitrarily set as 100%.--

Please replace paragraph **[62]** beginning at page 14, line 12, with the following:

--**[62]** Figure 10 illustrates schematic diagram of deletion constructs in C-terminal charged tails (SEQ ID NOS:16 and 17) of C14 and C370 sequences. LPQTG = (SEQ ID NO:13).--

Please replace paragraph **[73]** beginning at page 17, line 2, with the following:

--**[73]** The sequence LPQ(S/A/T)(G/A) acts as a cell wall sorting signal in vaginally associated strains of Lactobacillus. At least one copy of the motif LPQ(S/A/T)(G/A) is in the cell wall targeting region. The parentheses in the motif indicate alternative amino acids in

that position (*e.g.*, LPQSG, LPQAG, LPQTG, LPQSA, LPQAA, LPQTA (SEQ ID NOS:11, 12, 13, 18, 19 and 14, respectively)).--

Please replace paragraph **[74]** beginning at page 17, line 9, with the following:

--**[74]** The carboxyl terminus of a polypeptide to be anchored in the cell wall comprises a hydrophobic region that functions to span the bacterial membrane. The hydrophobic region comprises at least about 50%, and in some embodiments, at least 60%, 70%, 80% or 90% hydrophobic amino acids. Naturally occurring hydrophobic amino acids include alanine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan and valine. Some less hydrophobic amino acids, including glycine, threonine, and serine, can also constitute part of these sequences (*see, e.g.*, Pallen *et al.*, *Trends Microbiol.* 9:97-101 (2001)). Hydrophobic sequences generally are between about 10 and about 30 amino acids and sometimes 13 and 24 amino acids in length among available LPXTG (SEQ ID NO:9) -containing substrates for sortase-like proteins (Pallen *et al.*, *Trends Microbiol.* 9:97-101 (2001)). Exemplary hydrophobic sequences include, *e.g.*, V^{1740}GILGLAIATVGSLLGLGV^{1758} (SEQ ID NO:20) in C14 and P^{1877}LTAIGIGLMALGAGIFA^{1894} (SEQ ID NO:21) in C370.--

Please replace paragraph **[76]** beginning at page 17, line 28, with the following:

--**[76]** A charge region can be optionally present at the carboxyl terminus of a cell wall targeted protein, typically immediately following the hydrophobic membrane spanning region. The presence of a carboxyl terminal charged region anchors the polypeptide to the membrane, thereby greatly reducing the amount of protein that dissociates from the membrane and escapes into the media. The charged region comprises at least 40%, and in some embodiments, at least 50%, 60%, 70%, 80% or 90%, charged amino acids. Naturally occurring charged amino acids include arginine, histidine, lysine, aspartic acid and glutamic acid. Charged sequences can be between, *e.g.*, 2 and 20 amino acid residues and in some embodiments are

between 4 and 12 or between 5 and 11 amino acids in length. Exemplary charged sequences include, *e.g.*, $K^{969}KRKED^{974}$ (SEQ ID NO:22) in C191, $R^{1760}KKRQK^{1765}$ (SEQ ID NO:23) in C14, and $K^{1895}KKRKDDEA^{1903}$ (SEQ ID NO:24) in C370.--

Please replace paragraph **[92]** beginning at page 21, line 12, with the following:

--**[92]** A variety of signal sequences are known to direct expression of polypeptides to the membrane, extracellular space or the cell wall (*e.g.*, by covalent attachment to peptidoglycan). Exemplary signal sequences include the signal sequence from α-Amylase of *L. amylovorus* (Giraud & Cuny, *Gene.* 198:149-157 (1997)) or the signal sequence from the S-layer gene (*cbsA*) of *L. crispatus* (*e.g.*, MKKNLRIVSAAAAALLAVAPVAA (SEQ ID NO:25) or MKKNLRIVSAAAAALLAVATVSA (SEQ ID NO:26). Signal sequences are typically located at the amino-terminus of a polypeptide.--

Please replace paragraph **[121]** beginning at page 27, line 9, with the following:

--**[121]** The M6 proteins have a signature cell wall sorting signal, the LPXTG (SEQ ID NO:9) motif, followed by a stretch of hydrophobic amino acids and finally a sequence containing charged residues (KRKEEN; SEQ ID NO:10), which serves as a critical cell surface retention signal. We initially attempted a plasmid-based modular approach to express CD4 on the surface of *L. jensenii* by utilizing two well-characterized cell-wall anchor motifs, from either the M6 protein (*emm6*) of *S. pyogenes* or the PrtP protease of *L. paracasei*, or the anchor motif from the M6 protein of *S. pyrogenes* plus an N-terminal 100-amino acid extension (CWA100) derived from the native sequence of M6 protein. Unlike the M6 protein, the sorting signal for PrtP is LPKTA (SEQ ID NO:27). Western analysis of proteins in conditioned media and cell wall- or protoplast-associated protein pools in the modified *L. jensenii* harboring M6 or PrtP or CWA100 as cell wall anchors revealed no detectable cell wall associated 2D CD4, although

abundant 2D CD4 was released into conditioned media. Flow cytometric analysis failed to identify positive surface-exposed 2D CD4.--

Please replace the paragraph beginning at page 27, line 24, with the following:

--Database search of genomic sequences of *L. jensenii* allowed identification of approximately 30 contigs with putative cell wall anchor motifs. Based on a more detailed sequence homology search in the non-redundant databases available at the web site of the National Center for the Biotechnology, we selected three of these sequences, designated as C14, C191, and C370. They shared a low sequence similarity (with 23~27% identities) with Rlp of *Lactobacillus fermentum* (Turner *et al.*, *Appl. Environ. Microbiol.* 69:5855-5863 (2003)) or mucus binding protein in *L. reuteri* (Roos and Jonsson, *Microbiol.* 148:433-442 (2002)), a family of streptococcocal surface proteins (Wastfelt *et al.*, *J. Bio. Chem.* 271:18892-18897 (1996)), and a cell wall-anchored proteinase in *S. thermophilus* (Fernandez-Espla *et al.*, *Appl. Environ. Microbiol.* 66:4772-4778 (2000)), respectively. All of the three sequences have LPQTG (SEQ ID NO:13) sorting signal preceding a hydrophobic region and a charged C-terminal tail (See Figure 1). These features are common among sortase-recognized C-terminal cell wall anchor sequences in Gram-positive bacteria (Navarre and Schneewind, *Microbiol. Mol. Bio. Rev.* 63,174-229 (1999)). Among the LPXTG (SEQ ID NO:9) cell anchor motifs found in Gram-positive bacteria, only seven percent match the LPQTG (SEQ ID NO:13) sequence found in these *L. jensenii* proteins. C14, C191, and C370 proteins all contain tandem repeat domains adjacent to the cell wall anchor region, a structural feature that is frequently present in known cell wall anchored proteins (Navarre and Schneewind, *Microbiol. Mol. Bio. Rev.* 63:174-229 (1999)). The sequences of C14, C191 and C370 are displayed in Figure 2A-C.--

Please replace paragraph **[122]** beginning at page 28, line 11, with the following:

--**[122]** To determine the efficiency of C14, C191, and C370 to anchor heterologous fusion proteins to the cell wall of *L. jensenii*, we selected approximately 200 amino acids directly N-terminal to LPQTG (SEQ ID NO:13) sorting signal. This region, often defined as cell wall associated (CWA) domain in cell wall anchored proteins, may facilitate retention or extension of substrate sequence and thus proper proteolytic cleavage by membrane-associated sortase. To facilitate immuno-detection, c-Myc epitope (EQKLISEEDL; SEQ ID NO:15) was fused with the N-terminus of CWA200 regions of C14, C191, and C370 in pOSEL239, 240, and 241, respectively. Western and flow cytometric analyses were employed to investigate whether the c-Myc tagged proteins were produced and targeted to the cell wall. To perform Western analyses, the modified *L. jensenii* harboring pOSEL175, 239, 240, and 241 were grown in both MRS and Rogosa SL broth to logarithmic phase. Subsequently, the cell walls were digested with mutanolysin, an *N*-acetyl muramidase that cuts the β1-4 glycosidic bond between MurNAc-GlcNAc of the glycan strands in mature peptidoglycan. Cell wall anchored proteins typically migrate as a large spectrum of fragments, following SDS-PAGE chromatography (Perry *et al.*, *J. Biol. Chem.* 277, 16241-16248 (2002)). Western analysis of proteins in cell wall enriched fractions in the bacterial cells harboring pOSEL239 (C14 anchor) and 241 (C370 anchor) revealed a ladder of c-Myc tagged proteins on reducing SDS-PAGE when the bacterial cells were cultured in both MRS and Rogosa broth (Figure 3). These patterns were absent in the cell wall enriched fraction in the bacterial cells harboring pOSEL240 (C191 anchor), demonstrating different anchoring efficiencies among LPQTG (SEQ ID NO:13) -containing sequences tested.--

Please replace paragraph **[129]** beginning at page 31, line 9, with the following:

--**[129]** It is unclear whether a 36 amino acid C-terminal anchor motif, including LPQTG (SEQ ID NO:13) signal, a hydrophobic region, and a charged tail of C14 or C370 sequence would be sufficient to support efficient surface expression of 2D CD4 in the *L.*

jensenii. To address this question, two constructs, designated as pOSEL238 harboring the C-terminal anchor motif of C14 and pOSEL262 harboring the C-terminal anchor motif of C370 were prepared and analyzed in reference to negative controls pOSEL175 and 651, and positive control, pOSEL237. Western analysis of cell wall enriched fraction in *L. jensenii* harboring pOSEL238 after probing with pAb T4-4 detected no ladder patterns resembling those in pOSEL237. Furthermore, flow cytometric analysis of mAb Sim.4 binding to bacterial cells harboring pOSEL238 failed to detect any increase in mean fluorescence intensity relative to background control in cells harboring pOSEL175 (Figure 6). Similarly, FACS analysis of the bacterial cells harboring pOSEL262, in reference to those harboring pOSEL175 and positive control pOSEL249, yielded similar negative results. Consistent with these observations, surface expression of 2D CD4 was not achieved when similar length of C-terminal anchor motifs from *S. pyogenes* and *L. paracasei* were employed. This suggests that protein sequences upstream from the characteristic LPQTG (SEQ ID NO:13) motif contribute significantly to the cell wall anchoring process and are required to display biologically active proteins on the cell wall of *L. jensenii*.--

Please replace paragraph **[130]** beginning at page 31, line 30, with the following:

--**[130]** The native C370 sequence contains eight nearly identical tandem repeats, a characteristic of many cell wall anchor proteins in Gram-positive bacteria, in its C-terminal region upstream of the LPQTG (SEQ ID NO:13) motif (Figure 1). While two and half repeat sequences were included in the anchoring sequence of pOSEL249, it remains to be determined whether a different length of upstream sequence could be used to maximize surface protein display. Accordingly, several constructs were prepared harboring 0, 1, 2, 4, 7, and 8 repeats of the C370 sequence. They were designated as pOSEL262, 268, 278, 280, 281, 276, respectively. To determine level of 2D CD4 molecules that adopt a correctly folded conformation, the transformed bacteria were probed with mAb Sim.4 for flow cytometry analysis (Figure 7). There was non-distinguishable mean fluorescence intensity in bacterial harboring pOSEL262 (0 repeat)

from that in negative control pOSEL175, suggesting the requirement of repetitive sequence for proper surface expression of heterologous proteins. In addition, there was a significant increase in fluorescence intensity when number of repeats increased from 0 in pOSEL262 up to 3 in pOSEL278. The fluorescence intensity remained steady with additional increase in number of repeats.--

Please replace paragraph **[132]** (and header) beginning at page 33, line 1, with the following:

--Effect of mutagenesis of LPXTG (SEQ ID NO:9) motif on surface expression of 2D CD4 in *L. jensenii*

[132] When protein A of *Staphylococcus aureus*, a well studied cell wall anchor protein, was mutated on the LPETG (SEQ ID NO:28) cell wall sorting motif, it was found that replacing amino acid proline (P) in LPQTG (SEQ ID NO:13) with amino acid asparagine (N) decreased the efficiency of protein surface display, while replace threonine (T) with serine (S) had little effect on the efficiency of protein surface display (Navarre and Schneewind, *Microbiol. Mol. Biol. Rev.* 63:174-229 (1999)). This study indicated that the P residue is probably the most important residue in LPXTG (SEQ ID NO:9) motif, and the T residue can be replaced by a similar amino acid, S. To determine whether the LPQTG (SEQ ID NO:13) motif within the C14 and C370 is indeed the critical sorting signal, the importance of P and T within the LPQTG (SEQ ID NO:13) sequence was investigated. Point mutations were generated within the LPQTG (SEQ ID NO:13) motif by PCR on both C14 and C370 sequences. The P residue was mutated to alanine (A) or asparagine (N); the amino acid T was mutated to A, S or glycine (G); the amino acid G in the LPXTG (SEQ ID NO:9) motif was mutated to A. Plasmids with the altered LPQTG (SEQ ID NO:13) motif were designated as pOSEL237P(A), pOSEL237P(N), pOSEL237T(A), pOSEL237T(G), pOSEL237T(S), pOSEL237G(A), pOSEL249P(A), pOSEL249P(N), pOSEL249T(A), pOSEL249T(G), pOSEL249T(S), and pOSEL249G(A), respectively. Western and flow cytometric analyses of the *L. jensenii* 1153 harboring the mutated constructs were performed. Compared to the *L. jensenii* harboring parental pOSEL237

and pOSEL249, those harboring pOSEL237P(A), pOSEL237P(N), pOSEL249P(A), and pOSEL249P(N) did not exhibit the characteristic higher molecular weight species spectra, upon Western blotting of cell wall enriched protein fractions with pAb T4-4. Instead, there was a marked increase in secretion of 2D CD4-CWA200 fusion protein into the conditioned medium, indicating that the 2D CD4-CWA200 fusion proteins were not covalently linked to the cell wall. A characteristic spectra of higher molecular weight species, similar to those observed with wild type pOSEL237 and pOSEL249, was evident upon cell wall digestion of *L. jensenii* harboring pOSEL237T(S) and pOSEL249T(S), suggesting that the amino acid T within LPQTG (SEQ ID NO:13) from C14 and C370 can be effectively replaced by S (data not shown).--

Please replace paragraph **[133]** beginning at page 33, line 29, with the following:

--**[133]** To further determine the effect of mutagenesis of LPXTG (SEQ ID NO:9) on *L. jensenii* surface protein display, the *L. jensenii* strains harboring pOSEL175, 651, 237, 249, along with the various mutant constructs, were probed with pAb T4-4 or mAb Sim.4, and subsequently analyzed for antibody binding by flow cytometry. There was a substantial decrease of mean fluorescence intensity in bacterial cells harboring pOSEL237P(A), pOSEL237P(N) compared to pOSEL237, and for pOSEL249P(A), pOSEl249P(N) comparing to those harboring pOSEL249, indicating that there was much less 2D CD4 protein displayed on the cell surface, if any. However, the mean fluorescence intensity in the bacterial cells harboring pOSEL237T(S), pOSEL 237 (T)A, pOSEL249T(S), and pOSEL249 (T)A was comparable to *L. jensenii* harboring pOSEL237 and 249, demonstrating that replacing T with S or A has little effect on the efficiency of cell wall anchoring (Figure 9).--

Please replace paragraph **[134]** beginning at page 34, line 6, with the following:

--**[134]** The data from Western blot and flow cytometric analysis indicate that the amino acid P contained within LPQTG (SEQ ID NO:13) motif of C14 and C370 can not be

readily substituted. In contrast, the amino acid T can be replaced with S or A, yielding a protein that still anchors efficiently to the cell wall of *Lactobacillus*.--

Please replace paragraph **[135]** beginning at page 34, line 13, with the following:

--**[135]** One of the characteristics of gram-positive cell wall anchor domains is the stretch of positive charged amino acids at the extreme C-terminus of the protein. In the M6 proteins, this sequence (KRKEEN; SEQ ID NO:10) serves as a critical cell surface retention signal. These signature sequences have been found in other Gram-positive bacteria including *Staphlyococcus*, *Enterococcus*, *Listeria*, and *Lactobacillus* (Navarre and Schneewind, *Microbio. Mol. Biol. Rev.* 63:174-229 (1999)). Two sequences RKKRQK^{1765} (SEQ ID NO:23) and KKKRKDDEA^{1903} (SEQ ID NO:24) were identified as the positive charged tails in C14 and C370 putative anchor sequences respectively (Figure 1). To determine whether theses two sequences serve as cell surface retention signal, a series of deletion constructs were created (Figure 10). They were designated as pOSEL237-5, pOSEL237-6, pOSEL237-7, pOSEL249-8, pOSEL249-9, and pOSEL249-10, respectively.--

Please replace paragraph **[138]** beginning at page 35, line 9, with the following:

--**[138]** While most cell wall anchored proteins from Gram-positive bacteria share the same sorting signal LPXTG (SEQ ID NO:9), some of the proteins, however, have different motifs. The sorting signal for PrtP of *L. paracasei*, for example, is LPKTA (SEQ ID NO:27) (Holck and Naes. *J. Gen. Microbiol.* 138:1353-1364 (1992)). Protein L and the human serum albumin binding protein of ~~*peptostreptococcus*~~ *Peptostreptococcus magnus* share a motif of LPXAG (SEQ ID NO:29) (de Château & L. Björck. *J. Biol. Chem.* 269:12147-12151 (1994); Keller *et al.*, *EMBO J.* 11:863-874 (1992); Murphy *et al. DNA Seq.* 4: 259-265 (1994)). When LPQTG (SEQ ID NO:13) mutated to LPQAG (SEQ ID NO:12) or LPQSG (SEQ ID NO:11) in C14 or C370 anchor proteins, there was only a slight decrease in surface display of 2D CD4, as

measured by flow cytometry or Western blotting following SDS-PAGE. However, these sequences alone are insufficient to anchor proteins to the cell wall of vaginally derived lactobacilli as based on the following evidence: 1) the 36-amino acid C-terminal anchoring domain alone did not anchor c-Myc epitope, or 2D CD4 to the cell surface, 2) the prototypical M6 cell wall anchor sequence (encoded by the *emm6* gene of *S. pyogenes*) did not anchor heterologous proteins to the cell wall of vaginally derived lactobacilli, even when upstream sequences of up to 200 amino acids are included (we found a similar result when using the LPXTA (SEQ ID NO:30) motif from *L. paracasei*), and 3) the C191 protein was not an efficient anchor. These findings demonstrate that other upstream sequences contained within the CWA200 region of C14 and C370, also contribute significantly to the cell wall anchoring process.--

Please replace paragraph **[144]** beginning at page 38, line 4, with the following:

--**[144]** Cell wall anchored proteins of Gram-positive bacteria have a conserved C-terminal LPXTGX (SEQ ID NO:31) motif (Fischetti *et al.*, *Mol. Microbiol.* 4:1603-1605 (1990)). This hexapeptide is followed by a hydrophobic stretch of amino acids and a short charged tail, also known as a stop transfer sequence. (Schneewind *et al.*, *Cell* 70:267-281 (1992). In addition, another unique LPXTA (SEQ ID NO:30) sorting motif was identified in *Lactobacillus paracasei* (Holck and Naes., *J. Gen. Microbial.* 138:1353-1364 (1992)). To identify native cell wall anchor sequences, a computer script was written to identify motifs similar to LPXTG (SEQ ID NO:9) and LPXTA (SEQ ID NO:30) in all reading frames of the assembled contigs (resulting from estimated 75% complete genome sequence of *L. jensenii* 1153). The resulting contigs with putative cell wall anchor motifs were further verified by BLAST search for sequence homology to cell wall-anchored proteins in Gram-positive bacteria.--

Please replace paragraph **[146]** beginning at page 38, line 27, with the following:

--**[146]** To conveniently surface anchor proteins in *L. jensenii*, an expression cassette was constructed and sub-cloned into the *Sac*I and *Xba*I sites of pOSEL175. The cassette contains four components, including a lactobacillus-compatible P_{23} promoter, CbsA signal sequence of *L. crispatus*, DNA encoding a heterologous protein, and covalent cell wall anchoring domains from known or putative cell surface proteins in Gram-positive bacteria. Our detailed analyses of constructs harboring a series of promoters and signal sequences indicated that a combination of the P_{23} promoter from *Lactococcus lactis* (van der Vossenet *et al.*, *Appl. Environ. Microbiol.* 53:2452-2457 (1987)) and the signal sequence from the CbsA of *L. crispatus* (CbsAss) drives the highest levels of protein expression of 2D CD4 in the construct designated as pOSEL651 (Chang *et al.*, *Proc. Natl. Acad. Sci. USA*. 100:11672-11677 (2003)). Unique restriction sites, including *Sac*I, *EcoR*I, *Nhe*I, *Mfe*I, and *Xba*I were placed between each component from 5' to 3' ends, respectively. Amplification of each component by PCR was performed using *Pfu* DNA polymerase. Oligonucleotide primers for PCR amplification of various portions of the fusion constructs detailed in this study include the following:

P23.f	5'-GTGGAGCTCCCCGAAAAGCCCTGACAACCC-3' (SEQ ID NO:32)
P23.r	5'-GGAAACACGCTAGCACTAACTTCATT-3' (SEQ ID NO:33)
2DCD4.f	5'-GCGGCTAGCAAGAAAGTTGTTTTAGGTAAA-3' (SEQ ID NO:34)
2DCD4.r	5'-GCACAATTGTGATGCCTTTTGAAAAGCTAA-3' (SEQ ID NO:35)
CbsAss.f	5'-GCGAATTCAAGGAGGAAAAGACCACAT-3' (SEQ ID NO:36)

CbsAss.r 5'-[[1]]CCAGCTAGCTGAAACAGTAGAAACGGC-3' (SEQ ID NO:37)--

Please replace paragraph **[147]** beginning at page 39, line 16, with the following:

--**[147]** Proteins designed for surface expression include a 10-amino acid c-Myc peptide (EQKLISEEDL; SEQ ID NO:15) and the first 183 residues comprising the N-terminal two extracellular domains of human CD4 (2D CD4). The 2D CD4 protein was recoded to conform to a preferred lactobacillus codon usage. All expression constructs were confirmed by DNA sequence analysis prior to transformation into *L. jensenii*.--

Please replace paragraph **[148]** beginning at page 39, line 23, with the following:

--**[148]** We chose initially epitope tagging to determine the level of protein expression and whether it is feasible to use a defined length of putative cell wall anchor sequence for surface display of biologically active proteins. In order to not disrupt functioning of C-terminal sorting motif, oligonucleotide primers containing the 10 amino acid c-Myc epitope (EQKLISEEDL; SEQ ID NO:15) in the 5' end were designed, allowing fusion of c-Myc epitope to the N-terminus of the putative cell wall anchor sequences, including C14, C191, and C370 from the genome of *L. jensenii* 1153. The c-Myc sequences were either fused directly to the cell wall anchor motif of these proteins (the C-terminal 30 amino acids of C14, C191, and C370) or to sequences containing the C-terminal cell wall anchor domain and various lengths of contiguous upstream amino acids. Most notably, c-Myc was fused to a 200 amino acid sequence containing the cell wall anchor domain and upstream amino acids (designated CWA 200).

Myc14nhe (5' primer) (SEQ ID NO:38)

(GCGCTAGCGAACAGAAACTGATCTCCGAAGAGGACCTGGTAACTCGTACTATCAATGTA)

Myc14mfe (3' primer) (SEQ ID NO:39)

(CGCCAATTGCTACTTTTGACGTTTCTTTCT)

Myc191nhe (5' primer) (SEQ ID NO:40)

(GCGCTAGCGAACAGAAACTGATCTCCGAAGAGGACCTGGACGTAGTA ATTCCAGGAA)

Myc191mfe (3' primer) (SEQ ID NO:41)

(GCGCAATTGTTAATCTTCTTTTCTCTTCTT)

Myc370nhe (5' primer) (SEQ ID NO:42)

(GCGCTAGCGAACAGAAA CTGATCTCCGAAGAGGACCTGTTGAAGAAGGCAGAAGAAGT)

Myc370mfe (3' primer) (SEQ ID NO:43)

(CCGCAATTGTTATGCTTCATCATCTTTTCT)--

Please replace paragraph **[150]** beginning at page 40, line 24, with the following:

--**[150]** Three putative surface proteins containing C-terminal LPQTG (SEQ ID NO:13) anchoring motif were chosen to determine their ability to express foreign proteins on the cell wall of *L. jensenii* 1153. The DNA regions containing the C-terminal LPQTG (SEQ ID NO:13) domain and their upstream 200 amino acids of these surface proteins (tentatively designated as CWA200 region) were amplified by three sets of primers, as described below,

C14: 5' primer (GCGCAATTGGTAACTCGTACTATCAATGTA; SEQ ID NO:44)

3' primer (CGCTCTAGATACACAAACTATTTTACGGTC; SEQ ID NO:45)

C191: 5' primer (GCGCAATTGGACGTAGTAATTCCAGGAACA; SEQ ID NO:46)

3' primer (CGGTCTAGACCAAGCAATTTATATATTGCT; SEQ ID NO:47)

C370: 5' primer (GCGCAATTGAAGAAGGCAGAAGAAGT; SEQ ID NO:48)

3' primer (CCGTCTAGATTATGCTTCATCATCTTTTCT; SEQ ID NO:49)--

Please replace paragraph **[151]** beginning at page 41, line 1, with the following:

--**[151]** The internal *Mfe*I site of C14 anchor domain and the internal *Xba*I site of the C370 domain were mutated by site-directed mutagenesis before enzymatic restriction. All the PCR products of predicted size were gel-purified and digested with both *Mfe*I and *Xba*I. The resulting fragments were ligated with *Mfe*I/*Xba*I double digested pOSEL651, which contains P23-regulated secreted 2D CD4, to make plasmid pOSEL237 (via CWA200 of C14 sequence), pOSEL242 (via CWA200 of C191 sequence) and pOSEL249 (via CWA300 of C370 sequence), respectively. Alternatively, the C-terminal 36-amino acid anchor motif of C14 sequence was similarly cloned into shuttle vector by using following two primers.

Mfec14up: 5' GCGCAATTGCCACAAACTGGTTCTAAGACT (SEQ ID NO:50)

Xnac14lo: 3' primer (CGCTCTAGATACACAAACTATTTTACGGTC; SEQ ID NO:51)--

Please replace paragraph **[152]** beginning at page 41, line 12, with the following:

--**[152]** All of the resulting plasmids after verification of DNA sequences were electroporated into ~~*L. jesneii*~~ *L. jensenii*, *L. gasseri,* and *L. casei*.--

Please replace paragraph **[153]** beginning at page 41, line 16, with the following:

--**[153]** Different repetitive cell wall spanning regions upstream the C370 LPQTG (SEQ ID NO:13) motif were amplified from the genomic DNA of *L. jensenii* 1153. The same 3' primer (5'-CCGTCTAGATTATGCTTCATCATCTTTTCT-3'; SEQ ID NO:49) was used, in pair with the following 5' primers for each PCR reaction.

Zero repeat: 5'-CGGCAATTGCCTCAAACTGGTACTGA-3' (SEQ ID NO:52)

One repeat: 5'-CGGCAATTGGGTCAAACTACAAATAAAGAT-3' (SEQ ID NO:53)

Two repeats: 5'-CGCCAATTGGGTCAAACTACTGATAAGAGT-3' (SEQ ID NO:54)

Three repeats: 5'-GCGCAATTGGGTCAAACTACAAATAAAGAT-3' (SEQ ID NO:55)

Four-eight repeats: 5'-CGGCAATTGGGTCAAACTACTGACAAGAGC-3' (SEQ ID NO:52)

Both *Mfe*I and *Xba*I sites in these primers are underlined.--

Please replace paragraphs **[156]** through **[158]** beginning at page 42, line 18, with the following:

--**[156]** Point mutations were generated using QuickChange® XL Site-Directed Mutagenesis Kit from Stratagene (La Jolla, CA). Plasmid pOSEL237 (expressing 2D CD4 anchored via CWA200 of C14 sequence) and plasmid pOSEL249 (expressing 2D CD4 anchored via CWA200 of C370 sequence) were used as templates. The mutagenic primers were designed based on the nucleotide sequences corresponding to LPQTG (SEQ ID NO:13) and its flanking sequences on C14 and C370:--

~~[157]~~ C14-GAAAGTAAGAAGACTTTACCACAAACTGGTTCTAAGACTGAA (SEQ ID NO:57)

~~[158]~~ C370-CATAAGCAAACTCTATTGCCTCAAACTGGTACTGAAACTAACCCAC (SEQ ID NO:58)

Please replace paragraph **[159]** beginning at page 42, line 26, with the following:

--**[159]** The replacement nucleotides were selected using *L. jensenii* 1153 preferred codons:

237P(A): Proline on LPQTG (SEQ ID NO:13) of C14 was replaced with ~~Alaine~~ Alanine

5'-GAAAGTAAGAAGACTTTAGCACAAACTGGTTCTAAGA-3' (SEQ ID NO:59)

5'-GTCTTAGAaccAGTTTGTGCTAAAGTCTTCTTACTTTC-3' (SEQ ID NO:60)

237P(N): Proline on LPQTG (SEQ ID NO:13) of C14 was replaced with ~~asparagine~~ Asparagine

5'-GAAAGTAAGAAGACTTTAAATCAAACTGGTTCTAAGAC-3' (SEQ ID NO:61)

5'-GTCTTAGAACCAGTTTGATTTAAAGTCTTCTTACTTTC-3' (SEQ ID NO:62)

237T(A): Threonine on LPQTG (SEQ ID NO:13) of C14 was replaced with Alanine

5'-AGAAGACTTTACCACAAGCTGGTTCTAAGACTGAAC-3' (SEQ ID NO:63)

5'-GTTCAGTCTTAGAACCAGCTTGTGGTAAAGTCTTCT-3' (SEQ ID NO:64)

237T(G): Threonine on LPQTG (SEQ ID NO:13) of C14 was replaced with Glycine

5'-AGAAGACTTTACCACAAGGTGGTTCTAAGACTGAAC-3' (SEQ ID NO:65)

5'-GTTCAGTCTTAGAACCACCTTGTGGTAAAGTCTTCT-3' (SEQ ID NO:66)

237T(S): Threonine on LPQTG (SEQ ID NO:13) of C14 was replaced with Serine

5'-AGAAGACTTTACCACAAAGTGGTTCTAAGACTGAAC-3' (SEQ ID NO:67)

5'-GTTAGTTTCAGTACCACTTTGAGGCAATAGAGTTTG-3' (SEQ ID NO:68)

237G(A): Glycine on LPQTG (SEQ ID NO:13) of C14 was replaced with ~~Alanie~~ Alanine

5'-GACTTTACCACAAACTGCTTCTAAGACTGAACAAG-3' (SEQ ID NO:69)

5'-CTTGTTCAGTCTTAGAAGCAGTTTGTGGTAAAGTC-3' (SEQ ID NO:70)

249P(A): Proline on LPQTG (SEQ ID NO:13) of C370 was replaced with ~~Alaine~~ Alanine

5'-CATAAGCAAACTCTATTGGCTCAAACTGGTACTGAAAC-3' (SEQ ID NO:71)

5'-GTTTCAGTACCAGTTTGAGCCAATAGAGTTTGCTTATG-3' (SEQ ID NO:72)

249P(N) Proline on LPQTG (SEQ ID NO:13) of C370 was replaced with Asparagine

5'-CATAAGCAAACTCTATTGAATCAAACTGGTACTGAAAC-3' (SEQ ID NO:73)

5'-GTTTCAGTACCAGTTTGATTCAATAGAGTTTGCTTATG-3' (SEQ ID NO:74)

249T(A) Threonine on LPQTG (SEQ ID NO:13) of C370 was replaced with Alanine

5'-CAAACTCTATTGCCTCAAAGTGGTACTGAAACTAA-3' (SEQ ID NO:75)

5'-GTTAGTTTCAGTACCAGTTTGAGGCAATAGAGTTTG-3' (SEQ ID NO:76)

249T(G) Threonine on LPQTG (SEQ ID NO:13) of C370 was replaced with Glycine

5'-CAAACTCTATTGCCTCAAGGTGGTACTGAAACTAAC-3' (SEQ ID NO:77)

5'-GTTAGTTTCAGTACCACCTTGAGGCAATAGAGTTTG-3' (SEQ ID NO:78)

249T(S) Threonine on LPQTG (SEQ ID NO:13) of C370 was replaced with Serine

5'-CAAACTCTATTGCCTCAAAGTGGTACTGAAACT-3' (SEQ ID NO:79)

5'-GTTAGTTTCAGTACCACTTTGAGGCAATAGAGTTTG-3' (SEQ ID NO:80)

249G(A) Glycine on LPQTG (SEQ ID NO:13) of C370 was replaced with Alanine

5'-CTCTATTGCCTCAAACTGCTACTGAAACTAACCCAC-3' (SEQ ID NO:81)

5'-GTGGGTTAGTTTCAGTAGCAGTTTGAGGCAATAGAG-3' (SEQ ID NO:82)--

Please replace paragraph **[162]** beginning at page 44, line 11, with the following:

--**[162]** A series of deletion mutants, in which positively charged amino acid located at the C-terminus of C14 and C370 were generated by PCR amplification. Plasmids pOSEL237 and pOSEL249 were used as template. An oligonucleotide complementary to 2D CD4 sequence on pOSEL237 and pOSEL249 (CD4F 5'-GATCGTGCTGATTCACGTCGT-3'; SEQ ID NO:83) was used as forward primer. The following oligonuclotides (with restriction sites underlined) were used as reverse primers for amplifying the C-terminal of 2D CD4 cDNA and complete C14 and C370 CWA200 sequences:

C14-7	5'-GCGCTCTAGACTAAACACCTAAGCCTAATAAGC-3' (SEQ ID NO:84)
C14-6	5'-GCGCTCTAGACTAGTTAACACCTAAGCCTAATAAG-3' (SEQ ID NO:85)
C14-5	5'-GCGCTCTAGACTATCTGTTAACACCTAAGCC-3' (SEQ ID NO:86)
370-10	5'-GCGCTCTAGATTAAAAAATTCCTGCGCCTAATG-3' (SEQ ID NO:87)
70-9	5'-GCGCTCTAGATTATGCAAAAATTCCTGCGCCTAATG-3' (SEQ ID NO:88)
370-8	5'-GCGCTCTAGATTACTTTGCAAAAATTCCTGCGCC -3' (SEQ ID NO:89)--

Please insert the accompanying paper copy of the Sequence Listing, page numbers 1 to 39, at the end of the application.